Department of Genetics, Faculty of Medicine, Osaka Univ., Osaka, Japan

May 3, 1956

Prof. J. Lederberg
Department of Genetics,
University of Wisconsin,
Madison 6, Wisconsin, W.S. A.

Dear Professor Lederberg :-

As I do not touch directly upon the problem of elimination of F factor in E. coli K 12, I delivered your letter into Mr. Hirota's hand. He will send you a copy of his manuscript entitled "Artificial elimination of F factor in Bact. coli K 12" and a reprint of his paper concerning this problem, in a separate air mail. His manuscript mentioned above will be published in Nature before long.

Although you will see the detail in his original manuscript, some additional advices will be shown here. In the direct method, in order to exclude the effect of medium, a modification method has been devised by Mr. Hirota; overnight peptone-glucose culture is washed and starved in saline at 37°C for an hour, then added Co++ solution in the final concentration 20 mM, The culture is incubated for 3 hours more, and then spread over the surface of nutrient agar.

As you suggested, the frequency of conversion varies according to the strain of E. coli used. For example, F factor in Y-40, F+Y-70, 58-161 and in some dutants derived from 58-161 (V1r, V2r, V6r,Sr) can easily be eliminated by the direct method, whereas, original K 12, W-1485 and Hfr are very stable against the direct method. However, by the resistant isolation method, Mr. Hirota obtained F- strains of original K 12 and W-1485. He will send you those strains with pleasure, if you wish to get them.

We do not know the detail of Mr. Richter's experiment, so that we can hardly point out the cause of his failure. But, it may be noteworthy that effects of Co on the growth inhibition and F elimination are influenced by quantities of metal chelating substances in the medium. For example, histidine, citrate and some polypeptides are easily chelated with Co<sup>++</sup>, and decrease the effect. It is, therefore, necessary to use various concentrations of Co in one experiment according to the constitution of the medium.

Very sincerely yours,

9. Rikkawa